Letter to the Editor

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Enzymatic isotope dilution mass spectrometry (IDMS) traceable serum creatinine is preferable over Jaffe in neonates and young infants

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To the Editor,

The use of creatinine (Scr) for estimates of glomerular filtration rate (GFR) in neonates is hampered by both physiologic fluctuations and analytic aspects [1–5]. Postnatal life is characterized by a Scr rise, most pronounced in the most immature neonates, followed by a decrease, slowest in the most immature [1, 2]. Besides perinatal physiologic fluctuations, measurements also depend on the quantification method [3–5]. Initially, Scr reference values were based on uncompensated Jaffe, a colorimetric reaction using alkaline picrate. Jaffe assays suffer from interference by endogenous (e.g., pseudocreatinines, hemoglobin F, IgG, bilirubin) and exogenous (e.g., cephalosporins) substances commonly retrieved in neonatal samples [3–5]. More recently, enzymatic methods were introduced. These assays are less prone to such interferences and thus more suitable in neonates. Nevertheless, enzymatic assays can also be affected by interferences (e.g., bilirubin, dopamine) [3–7]. It is generally accepted that uncompensated Jaffe overestimates Scr and fixed corrections (e.g., 0.2 or 0.3 mg/dL) or adaptations in the analytical procedure (e.g., rate blanking) have been suggested to adapt Jaffe assays observations [3–7]. To illustrate the limitations of any fixed correction, postnatal age-related between assay differences in the mean reference Scr values of uncompensated Jaffe or enzymatic Scr in two consecutive cohorts (2000–2005 vs. 2007–2010) of extreme preterm neonates varied between 0.12 and 0.27 mg/dL [2].

The Scr standardization program was created by the National Kidney Disease Education Program to reduce assay variation [6]. Jaffe and enzymatic quantification methods were calibrated to isotope dilution mass spectrometry (IDMS). We hypothesized that the IDMS-based Scr standardization program affects the extent of the remaining between assay Scr differences. To explore this and its covariates in neonates, Scr values measured by Jaffe and enzymatic methods in the same serum samples were compared.

Following approval (B322201214963, UZ Leuven, Belgium) and informed written parental consent, an additional blood sample (0.6 mL) was collected when sampling was performed for clinical indication. Each newborn was only included once. Samples were centrifuged (Eppendorf centrifuge 5415R, room temperature, 5 min, 5000 rpm, equal to 2320 *g*) and serum was subsequently divided in two containers and stored (–80°C) until analysis. Clinical characteristics [gestational age, postnatal age, postmenstrual age, birth weight, current weight, co-administration amoxicillin, co-administration dopamine, packed cells transfusion during neonatal stay before sampling, bilirubin (total, direct), and albumin] were registered. This list was based on the literature on in vitro interferences as described for creatinine assays and on the compounds prescribed [8]. Samples were analyzed by a Jaffe and an

Table 1 Clinical characteristics. Data provided by median and range, mean and standard deviation or incidence.

Gestational age (GA), weeks	38 (range 24–41)
Postnatal age, days	3 (range 0–236)
Postmenstrual age, weeks	39 (range 26–75)
Current weight, g	3190 (range 625–4550)
Preterm, <37 weeks GA	33%
Extreme preterm, <32 weeks GA	18%
Co-treated amoxicillin	11%
Co-treated dopamine	0%
Received packed cells transfusion	18%
Albumin, g/L	34.7 (SD 6.24)
Bilirubin, total, mg/dL	6.8 (SD 4.55)
Bilirubin, direct, mg/dL	0.53 (range 0.18–2.42)
Creatinine, Jaffe, mg/dL	0.50 (range 0.17–1.16)
Creatinine, enzymatic, mg/dL	0.49 (range 0.06–1.11)
Difference (Jaffe – enzymatic), mg/dL	0.013 (range –0.2 to 0.11)

enzymatic method, using methods currently available in the University Hospitals Leuven (i.e., Roche Diagnostics compensated Jaffe and enzymatic method, both IDMS traceable, measured on Cobas c702 module). Albumin and bilirubin (total and direct) were measured by bromocresol green and diazo colorimetric assays, respectively (Roche Diagnostics, Cobas c702 module). Clinical characteristics and bio-analytical results were described, regression and Bland-Altman analysis were performed to explore differences between both measurements and trends in its variability. Paired analysis (Wilcoxon) was performed to quantify differences between both analyses, and covariates of differences were explored (Rank correlation, Mann-Whitney U, linear regression).

Samples were collected in 129 neonates and young infants. Clinical characteristics and Scr values are provided in Table 1. A significant relationship between both Scr measurements was documented (r^2=0.9487, y=0.0038+0.967x, p<0.001). Bland-Altman analysis illustrates that there is no Scr concentration-related impact on this difference. The mean difference between both assays is 3.9%. In contrast, bilirubin (total, direct) had a significant effect on the difference between both assays (total bilirubin, p<0.0001). Jaffe creatinine was proportionally lower when compared to enzymatic measurement in the setting of elevated bilirubin (Figure 1). Similarly, a significant effect of albumin on the between assay difference was documented (p=0.0016). In a multiple regression model, only total bilirubin remained significant (r^2=0.24, p<0.001).

In essence, a statistical significant (p=0.0039) but clinical likely not very relevant mean difference of 0.013 mg/dL between IDMS traceable Jaffe and enzymatic assays was documented, and the extent of the difference was in part explained by bilirubin. There are several reports that illustrate that Jaffe as well as enzymatic measurements are affected by bilirubin, although interference of enzymatic methods has been minimized [7–9]. Hyperbilirubinemia (unconjugated) is common, because of age-related maturation of glucuronidation [10]. In the current study, rising bilirubin (up to 16.1 mg/dL) resulted in relatively lower (up

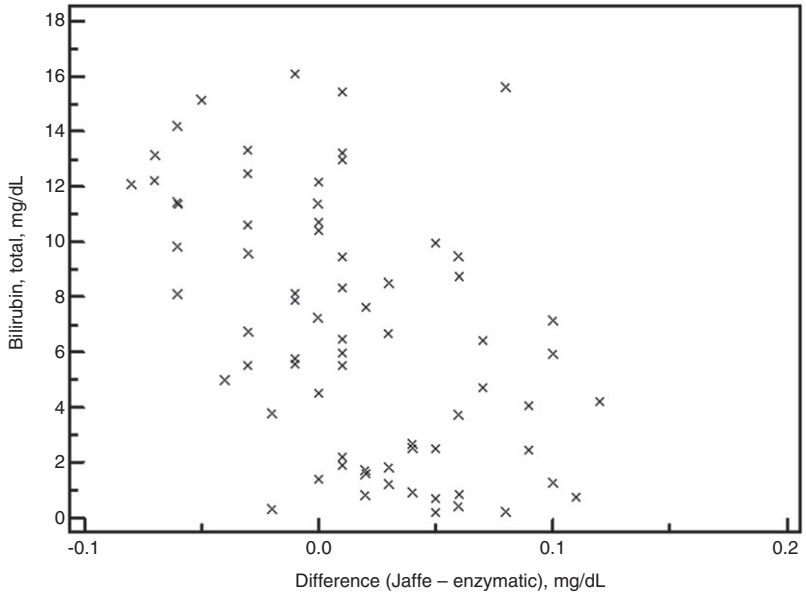

Figure 1 A significant correlation (rho=–0.52, 95% CI –0.67 to –0.32, p<0.0001) between total bilirubin and the absolute difference (Jaffe–enzymatic, mg/dL) in serum creatinine between both assays was observed.

to 0.1 mg/dL) Jaffe Scr values compared to enzymatic measurements (Figure 1). This is likely explained by overcorrection of the Jaffe assay. Bilirubin interference is caused by an oxidation of bilirubin to biliverdin in the alkaline Jaffe reaction medium. The Roche Jaffe assay uses rate-blanking to compensate for this interference. Prior to adding picric acid (and starting the reaction), alkaline is added to the sample, the bilirubin oxidation rate is measured and later on subtracted from the measured reaction rate of creatinine with alkaline picric acid. As the oxidation of bilirubin in alkaline is curvilinear, overcorrection can occur with increasing bilirubin. These observations are in agreement with the findings of Roche. Significant interference is excluded up to 10 mg/dL unconjugated bilirubin for the Jaffe and up to 20 mg/dL for the enzymatic assay. In clinical laboratory practice, Jaffe creatinine results from samples with high bilirubin concentrations (>10 mg/dL) should not be reported or a warning should be added. In addition, we confirmed an albumin concentration related effect on the difference between both assays, although the impact is more limited when compared to bilirubin. We initially aimed to compare both observations to a 'gold' standard reference for creatinine like high performance liquid chromatography (HPLC) or mass spectrometry. Unfortunately, because of insufficient remaining sample volumes, this was not feasible. Consequently, we can only describe differences between both assays used, and are unable to make suggestions on how these assays relate to such a gold standard or how other Jaffe or enzymatic assays relate to these findings [7]. Other limitations may be the absence of patients with very high bilirubin (>16 mg/dL), and the limited number of cases exposed to co-medication (antibiotics, inotropics).

Taking these limitations into account, differences between IDMS traceable Jaffe and enzymatic assays were limited, even in a heterogeneous group of neonates with still an assay-specific impact of bilirubin.

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Conflict of interest statement

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